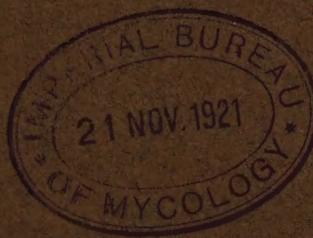


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On the Temporary Suspension of Vitality
in the Fruit-Bodies of Certain
Hymenomycetes.

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On the Temporary Suspension of Vitality in the Fruit-Bodies of Certain Hymenomycetes.

By A. H. REGINALD BULLER and A. T. CAMERON.

(Read May 14, 1912).

In 1909 Buller¹ showed that the fruit-bodies of a large number of species of Hymenomycetes which grow on sticks and logs, can be dried up without any loss of vitality, and that the vitality of some species, after desiccation has taken place, may be continued for years. When the fruit-bodies are moistened once more, after a few hours spore, liberation recommences. Streams of spores are emitted for several days, and the spores liberated from the revived fruit-bodies are capable of germination. Typical genera having fruit-bodies, the vitality of which will resist desiccation, are *Corticium*, *Stereum*, *Daedalea*, *Polystictus*, *Lenzites*, and *Schizophyllum*.

It was also shown by further investigation² that dried fruit-bodies, exposed to the air, lose their vitality in the course of a few months or years in the same manner as the seeds of Flowering Plants. Thus *Marasmius oreades* recovered on moistening, after desiccation for six weeks, but not after three months. *Lenzites betulina* recovered after desiccation for three years, but not after five years. From some recent experiments, here recorded for the first time, Buller has found that dried fruit-bodies of *Daedalea unicolor*, exposed to air in the dark, can retain their vitality for at least seven and one-half years, and those of *Schizophyllum commune* for at least five years and seven months. The fungi recover much more slowly for each year their desiccation is prolonged, whence it would appear that their vitality is becoming impaired; doubtless in the course of a few years it will be lost altogether. It is well known that many seeds behave in a similar manner; the vigour with which they germinate decreases year by year as desiccation is continued, until life becomes extinct.

The resemblance between the behaviour of seeds and the fruit-bodies of Hymenomycetes, in respect to the retention and the gradual loss of vitality in the desiccated condition, is sufficiently obvious. It has been shown by several investigators that many seeds retain their

¹ Buller, "Researches on Fungi," Longmans, Green & Co., London, 1909, pp. 105-111.

² Ibid. p. 111.

vitality *in vacuo*. Maquenne,¹ for instance, found that grains of wheat can withstand subjection to a vacuum of less than one-hundredth of a millimetre of mercury pressure for several months without losing their power of germination, and he suggested that under such conditions vitality may be completely suspended. The question of the entire suspension of vitality in the reproductive bodies of plants has been investigated anew in a very thorough manner by Paul Becquerel, who has published a series of papers on the subject in the "Comptes rendus." He first found² that dried seeds of various kinds, such as peas, on being placed in pure and dry nitrogen in the dark for a year, did not liberate a trace of carbon dioxide, and yet germinated subsequently. In later experiments³ he has shown that the seeds of Lucerne, and of White Mustard, and also grains of Wheat, after having their coats perforated, can be subjected to total desiccation in a vacuum of one five-hundredth of a millimetre pressure of mercury for one year, and that during this period they can be subjected for three weeks to the temperature of liquid air (-190° C.), and for seventy-seven hours to the temperature of liquid hydrogen (-253° C.), and that, nevertheless, when subsequently they are supplied with air and moisture at a suitable temperature, they germinate in a normal manner. Becquerel came to the conclusion that his experiments, along with those of his predecessors, indicate that at least for the seeds upon which he experimented, an interruption of life is not only possible but actual, without there being any indication of the existence of a limit to its prolongation. Still more recently⁴ he extended his investigations to the spores of certain moulds, (*Mucor mucedo*, *M. racemosus*, *Rhizopus niger*, *Sterigmatocystis nigra*, and *Aspergillus glaucus*). These were dried slowly, in the presence of caustic barya, for two weeks at 35° C. The containing tubes were afterwards sealed to a Berlement mercury pump and evacuated as perfectly as possible; they were sealed off after a McLeod guage indicated a pressure of less than one-thousandth of a millimetre of mercury. The vacuum was maintained for twenty-five months, and during this period the spores were subjected to the temperatures of liquid air and liquid hydrogen for three weeks and seventy-seven hours respectively, as was done with the seeds. When the spores were subsequently placed upon sterilized nutritive media, they soon germinated in a normal manner, and a fresh crop of spores was quickly produced.

¹ Maquenne, *Compt. rend.* 1902, T. 134, p. 1243.

² Paul Becquerel, "Sur la nature de la vie latente des graines et sur les véritables caractères de la vie," *Comp. rend.* 1906, T. 143, pp. 1177-9.

³ Paul Becquerel, "Sur la suspension momentanée de la vie chez certaines graines," *Comp. rend.*, 1909, T. 148, pp. 1052-4.

⁴ Paul Becquerel, "Recherches expérimentales sur la vie latente des spores des Mucorinées et des Ascomycetes," *Compt. rend.*, 1910, T. 150, pp. 1437-9.

Hitherto the material used for testing the latency of life in plants appears to have been limited to seeds and fungus spores. It seemed to the authors that it would be of interest to extend these investigations to the fruit-bodies of the Hymenomycetes, and the following is an account of the experiments we have made.

Some fruit-bodies of *Schizophyllum commune* were collected from stumps in a wood near Winnipeg in October, 1909. The material, which had recently developed when gathered, was brought to the laboratory, and allowed to dry by exposure to air. Experiments were begun on these fruit-bodies after they had been kept dry and exposed to ordinary air at room temperature for one year and two months. Their vitality was tested in the following manner. Some of them were placed in a damp-chamber, with wet cotton-wool on their upper surface. The two halves of each split gill re-apposed themselves to form complete gills,¹ and within twelve hours spore discharge had begun, and it continued for several days. The fact of spore-discharge was proved by the "beam of light" method,² which Buller has shown is of great convenience and certainty in testing for the retention of the vitality of fungus fruit-bodies. Spore-discharge is in itself an active vital process and the spores falling from such fruit-bodies readily germinate.

Others of the dried fruit-bodies were then tested for retention of vitality when completely dried and kept *in vacuo*. Each fruit-body was placed in a glass tube about 0.5 inch wide and 8 inches long, which had been previously closed at one end. A series of twelve such tubes were sealed to a phosphorus pentoxide tube in connection with a Töpler mercury pump—the von Antropoff³ modification. In order to complete the desiccation of the fruit-bodies, they were left in contact with the tube containing phosphorus pentoxide for twenty-four hours at a pressure of about 20 millimetres of mercury. The whole apparatus was then exhausted as completely as possible, and the twelve tubes were sealed off successfully at a vacuum of less than one-tenth of a millimetre pressure of mercury. The tubes were sealed up on December 10th, 1910, and were kept in the dark at room temperatures.

Up to the present time three of the tubes have been opened, one after one week, one after fourteen weeks, and one after one year and four and a half months. In each case the tube was joined to the evacuation apparatus by means of rubber pressure-tubing, the apparatus evacuated, and the end of the glass tube broken within the pressure tubing. A manometer, previously joined to the apparatus, showed no observable

¹ Cf. Buller, "Researches on Fungi," p. 117.

² Buller, loc. cit., pp. 94-101.

³ von Antropoff, "Eine vereinfachte und verbesserte Form der Töplerschen Quecksilberluftpumpe," Chem. Zeitschr., 1910, 34, 979.

change of pressure; this indicated that no appreciable amount of gas had been evolved.¹ The fruit-body was then removed from the tube, and on examination was found not to have altered in appearance: no darkening was observable. The fruit-body was then immediately placed in a damp-chamber, and tested by the beam-of-light method in the manner to which reference has been made already. In each case, at the end of some twelve hours, a dense and unbroken stream of spores was observed escaping from the under side of the fruit-body. The spore fall period lasted for about ten days. Some of the spores which had just fallen from the fruit-body taken from the third tube, were placed in nutrient gelatine. Many of them germinated within seventeen hours, and by the end of that time had produced germ-tubes longer than themselves. After three days the mycelium of each plant was considerably branched and extended. It was thus conclusively proved that a fruit-body of *Schizophyllum commune*, when kept thoroughly desiccated *in vacuo* for sixteen and one-half months, retains its vitality. This result is similar to that obtained by Becquerel for seeds and fungus spores.

It has been mentioned above that Becquerel not only subjected his material to perfect desiccation *in vacuo*, but also exposed it for some time to the extremely low temperatures of liquid air and liquid hydrogen. Unfortunately, owing to lack of the necessary facilities in Winnipeg, we have not found it possible to use these low temperatures.² However, we have been able to show that *Schizophyllum commune* is extremely resistant, not only to low temperatures, but also to rapid changes of temperature. Thus Buller³ gathered some fruit-bodies from a wood-pile in Winnipeg in the month of March at -17° C., after they had been exposed for several months to severe frost, the temperature having ranged for several weeks during that period between -15° and -40° C. After the fruit-bodies had been thawed for a few hours, the liberation of spores began anew, and very soon well marked spore-deposits were produced. The rise of temperature from -17° to a room temperature of about + 20° C. had been withstood successfully.

Two experiments of the reverse kind were carried out by us in the following manner:—

(1) On January 11th, 1911, which was a very cold day, a fruit-body, actively shedding spores in a warm room at a temperature of about 17° C., was suddenly placed in the open where the thermometer

¹ It is questionable whether a fungus *in vacuo* liberates any gas whatever; but this point can only be settled conclusively by using much larger quantities of material at any one time.

² *Vide Addendum.*

³ Buller, loc. cit., pp. 126-7.

registered a temperature of -31° C. After being exposed to this low temperature for three hours, the fruit-body was transferred to the warm room again; the gills were found to be cleft as a result of freezing; but, on thawing, the gill-halves rapidly approximated. For the first two hours after thawing no spores were liberated, but at the end of this period spores began to be shed in considerable numbers.

(2) Another fruit-body which was shedding spores, was *immersed in water*, and the containing vessel was set in the open during the whole of one night (11-12 January, 1911). The lowest temperature registered during this period was -35° C. In the morning the lump of ice containing the fruit-body was brought into the laboratory, and quickly thawed, and the resulting water was rapidly raised to a temperature of about $+40^{\circ}$ C. The fungus was then removed from the water: it commenced to shed spores in less than seven hours.

Buller's observations and these two experiments show how highly resistant *Schizophyllum commune* is to very rapid and very considerable changes of temperature. We have little doubt, although further experiment is necessary, that the fungus would, like seeds and the spores of Moulds, withstand exposure to the extremely low temperatures of liquid air and liquid hydrogen.

One further observation concerning desiccation may be recorded here. In nature the drying of an active fruit-body is of necessity comparatively slow, owing to slowness in changes of weather, and such drying, as field and laboratory experiments have shown, is never injurious. However, by extremely rapid and thorough artificial drying, it is possible to kill an active fungus. This we have proved by taking a fruit-body which was shedding spores, placing it while still thoroughly moist in a glass tube, sealing the glass tube in contact with a phosphorus pentoxide tube to a Töpler pump, and drying the fungus *in vacuo*. The pressure of water-vapour was reached in five minutes, and a pressure of less than a millimetre of mercury in one and a half hours. The dried fungus was removed after six hours, and moistened in the usual way in the damp-chamber, but it failed to recover. The gills turned dark brown and became putrid without shedding any spores. Such intensely rapid drying does not take place in nature, and it is not a matter for surprise to find that the fungus cannot withstand it.

It is possible that the vitality of seeds and Mould spores, and we may now add, certain hymenomycetous fruit-bodies, may be retained indefinitely, when they are thoroughly dried *in vacuo*, kept in the dark, and protected from exposure to injurious radiations. However, this conclusion can only be established by further experiment. It seems to us advisable that seeds, spores, and fruit-bodies should be studied, which normally retain their vitality, when dried and exposed to the air,

for conveniently short periods of time. If it should be found that the vitality of such plant structures when they are kept dry, *in vacuo*, in the dark, persists indefinitely, i.e., for many years longer than usual, then the hypothesis of entire suspension of vitality will receive strong support. If, on the other hand, experience should show that any seeds or spores or fruit-bodies, when kept under these conditions, nevertheless lose their vitality, positive evidence will be forthcoming that after all the vitality has not really been temporarily suspended. Our experiments with the unopened tubes of *Schizophyllum commune* still at our disposal, are planned to extend, if necessary, over a period of 25 years.

Summary of the Chief Results.

Fruit-bodies of *Daedalea unicolor* can retain their vitality when dried, kept in the dark, and exposed to ordinary air at room temperatures, for at least seven and a half years, and those of *Schizophyllum commune* for at least five years and seven months.

Fruit-bodies of *Schizophyllum commune*, after previous thorough drying by exposure to phosphorus pentoxide *in vacuo*, retain their vitality after being kept for sixteen and a half months in a vacuum at a pressure of not more than one-tenth of a millimetre of mercury, in the dark at room temperatures.

In their retention of vitality, when dried, exposed to ordinary air, or kept *in vacuo*, the fruit-bodies of certain Hymenomycetes resemble the seeds of Higher Plants, and the spores of Moulds.

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ADDENDUM.

Since the above was written some further experiments have been carried out at the University of Birmingham. It was found that fruit-bodies of *Schizophyllum commune* retain their vitality when dried, kept *in vacuo*, and at the temperature of liquid air for three weeks. A further account of these experiments will be given in another place.

A.H.R.B.

